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# Characterisation of rhamnolipid production in a previously un-investigated, non-pathogenic marine Pseudomonad.

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Arguably the most well characterised types of bacterially produced biosurfactants are the rhamnolipids (RL). Classically RL are know to be produced by the Gram negative opportunistic pathogen *Pseudomonas aeruginosa*. RLs possess a great deal of potential for commercial exploitation however their production by a species of bacterium pathogenic to humans limits this application. Therefore a number of studies have been aimed at identifying alternative, non-pathogenic RL synthesising organisms. **The aim of our study was to screen bacterial strains isolated from the marine environment for biosurfactant production; initial screening identified several strains of interest including strain MCTG214(3b1) which was shown to significantly reduce the surface tension of culture media following growth. Here we describe the characterisation of this bacterium and its products.**

MCTG214(3b1) was phylogenetically characterised initially by 16S rDNA sequencing and them by whole genome sequencing. This resulted in the identification of the strain as *Pseudomonas mendocina*. Measurement of cell free supernatant surface tension coupled with CFU counts obtained throughout a 72 h growth cycle using rapeseed oil as a carbon source showed a significant reduction in surface tension to take place during the exponential growth phase which leads to a sustained surface tension of approx. 32 $mNm^{-1}$ during stationary phase, **fig 1.** This pattern appeared highly similar to that previously observed in *P. aeruginosa* due to RL synthesis.

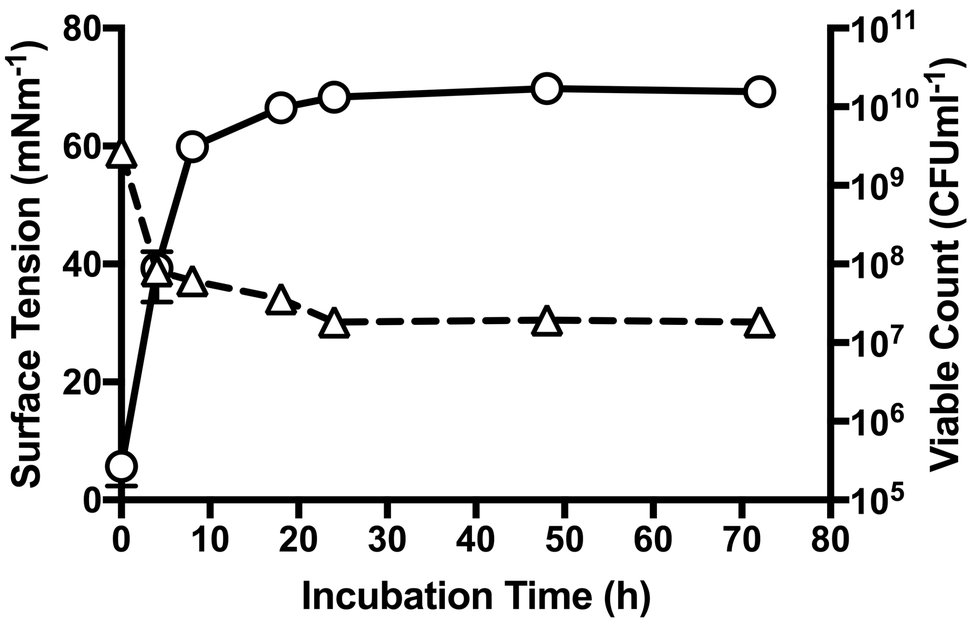



**Fig. 1.** CFU (○) and surface tension (△) measurements of MCTG214(3b1) cultures throughout growth in media with rapeseed oil as a carbon source.

The virulence of *Pseudomonas* sp. MCTG214(3b1) was assessed using the *Galleria mellonella* (Wax Worm) larvae infection model. Larvae inoculated with MCTG214(3b1) showed a 100% survival rate following 48 h of incubation at 37°C, larvae inoculated with the same infective dose of *P. aeruginosa* PAO1 were killed in under 24 h when incubated under the same conditions, **Fig 2**. Therefore in this model MCTG214(3b1) was shown to be significantly less pathogenic than *P. aeruginosa.*

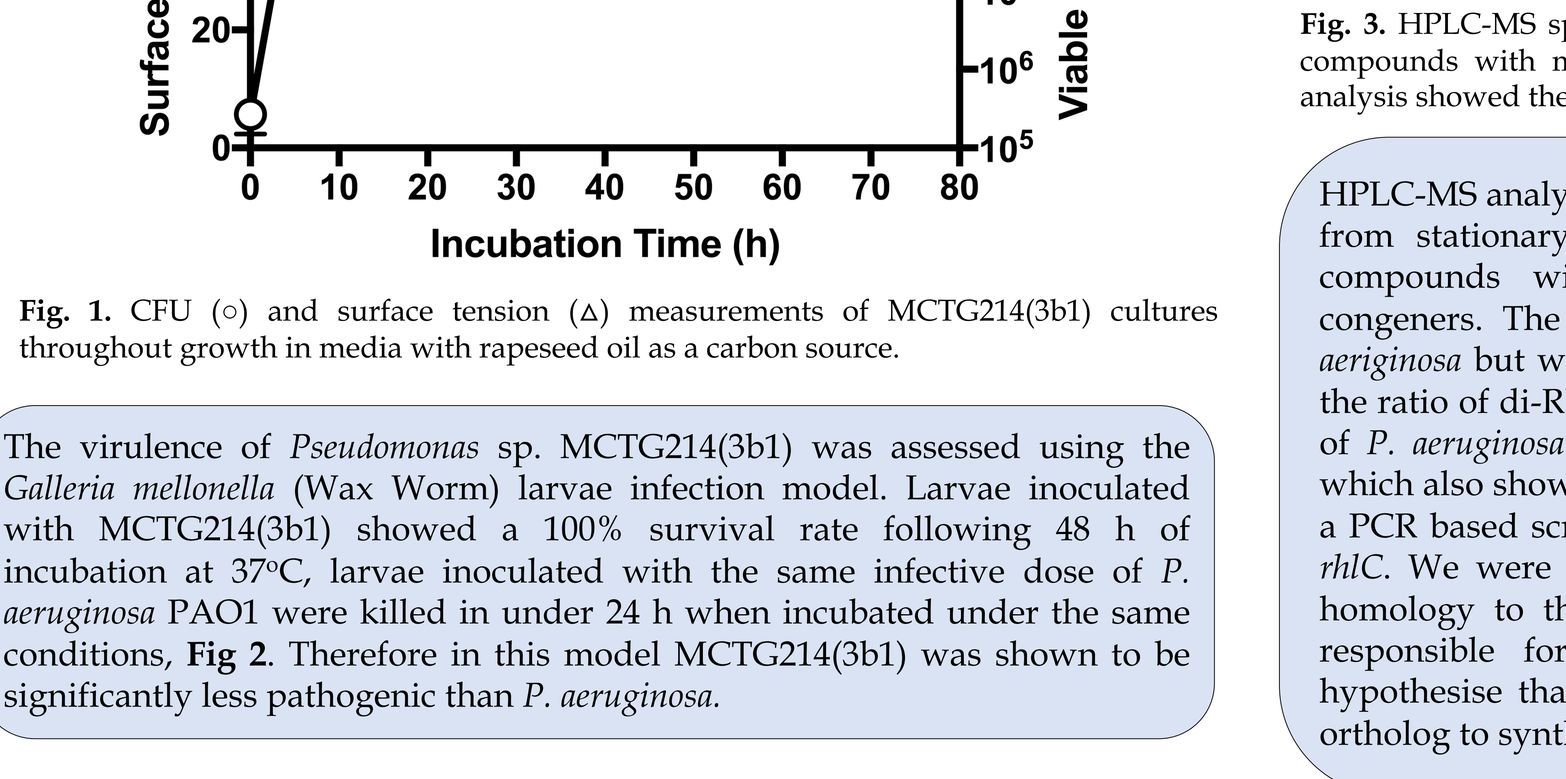
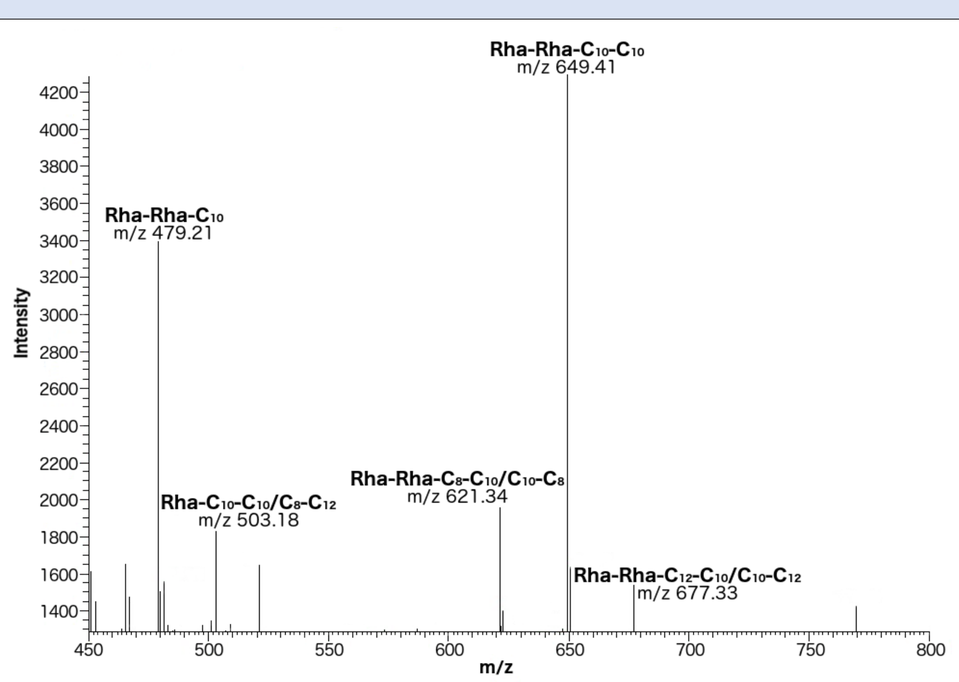



**Fig. 2.** Kaplan-Meier plot showing percentage survival of *Galleria mellonella* larvae after inoculation with either *Pseudomonas sp.* MCTG214(3b1) or *P. aeruginosa* PAO1. n = 30 (pooled from 3× duplicate experiments). Photo insert shows examples of live and dead *G. mellonella* larvae following infection with PAO1.




| RT min | m/z | Compound | $M_w$ | Mol. Form | Relative % |
|---|---|---|---|---|---|
| 13.31 | 479.21 | Rha-Rha-$C_{10}$ | 480.55 | $C_{22}H_{40}O_{11}$ | 23.80 |
| 13.36 | 649.41 | Rha-Rha-$C_{10}$-$C_{10}$ | 650.79 | $C_{32}H_{58}O_{13}$ | 42.74 |
| 14.29 | 503.18 | Rha-$C_{10}$-$C_{10}$/ $C_8$-$C_{12}$ | 504.65 | $C_{26}H_{48}O_9$ | 12.26 |
| 14.54 | 677.33 | Rha-Rha-$C_{10}$-$C_{12}$/ $C_{12}$-$C_{10}$ | 678.84 | $C_{34}H_{62}O_{13}$ | 9.78 |
| 32.31 | 621.34 | Rha-Rha-$C_8$-$C_{10}$/ $C_{10}$-$C_8$ | 622.74 | $C_{30}H_{54}O_{13}$ | 11.42 |

**Fig. 3.** HPLC-MS spectra of SPE purified MCTG214(3b1) supernatant extracts showing compounds with molecular weights correspondent to known RL congeners. Further analysis showed the percentage relative amounts of each congener (table).

HPLC-MS analysis of SPE purified, cell-free supernatant extracts generated from stationary MCTG214(3b1) cultures showed the presence of five compounds with molecular weights correspondent to known RL congeners. The RL congeners were similar to those synthesised by *P. aeriginosa* but were present at differing relative abundancies. Additionally the ratio of di-RL congeners to mono-RL was significantly higher than that of *P. aeruginosa*. This chemical analysis was reinforced by NMR results which also showed evidence of RL (data not shown). Finally we carried out a PCR based screen for orthologues to RL synthesis genes (*rhlA*, *rhlB* and *rhlC*. We were able to amplify and sequence genes with high levels of homology to the *rhlA* and *rhlB* genes of *P. aeruginosa*. The *rhlC* gene, responsible for di-RL synthesis still however remains elusive, we hypothesise that MCTG214(3b1) is utilising an as yet undiscovered *rhlC* ortholog to synthesis the di-RL that we observed in our chemical analysis.

**Conclusions**

- Marine bacterium *Pseudomonas sp.* MCTG214(3b1) is phylogenetically distinct from *P. aeruginosa*.
- Both phenotypic and subsequent chemical analysis have proved this strain is actively producing RL
- MCTG214(3b1) possesses *rhlA* and *rhlB* orthologues making it genetically capable of producing RL
- In comparison to *P. aeruginosa* MCTG214(3b1) is significantly less pathogenic.

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